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Synthesis and *in vitro* Antimycobacterial Activity of Ciprofloxacin Acetyl Isatin Derivatives

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Ten novel acetyl isatin derivatives of ciprofloxacin with marked improvement in lipophilicity, compared to the parent drug ciprofloxacin, were designed, synthesized and evaluated for their *in vitro* antimycobacterial activity against *M. smegmatis* and *M. tuberculosis* $H_{37}Rv$. All the targets (MIC: 2-128 µg/mL) exhibited inhibitory activity against the above bacterial strains. The most active compound **3h** (MIC: 2 µg/mL) was found to be two fold more potent than isoniazid and comparable to rifampin against *M. smegmatis*. Compound **3g** has a potency that of rifampin against *M. tuberculosis* $H_{37}Rv$. The result indicated that the lipophilicity of the tested targets is not the sole factor affecting the antimycobacterial activity.

Keywords: Ciprofloxacin acetyl derivatives, *Mycobacterium tuberculosis*, Isatin.

INTRODUCTION

Tuberculosis (TB), which is caused by mycobacteria, *M. tuberculosis* (MTB), is one of the most common and even fatal human diseases. It is estimated by WHO that there are millions of new tuberculosis patients every year throughout the world and one-third of the world population latently are infected with *M. tuberculosis* [1]. To make things worse, *M. tuberculosis* has evolved into emergence of multi-drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant strains and made the first-line anti-tuberculosis drugs, such as isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB) less effective to treat tuberculosis. Therefore, there is an urgent need to develop highly effective, novel anti-tuberculosis drugs with low toxicity profiles and effective to treat patients infected by drug-resistant tuberculosis strains [2].

Fluoroquinolones (FQs, **1**) have demonstrated potent anti-bacterial activity; some of them such as ciprofloxacin (CPFX) are currently recommended as the second-line therapeutics by WHO for the treatment of tuberculosis that shows resistance or tolerance to first-line antituberculosis therapies [3,4]. Fluoroquinolones exert their bacterial activity by interfering with function of two types of II bacterial topoisomerases, DNA gyrase (the main target in Gram-negative bacteria) and topoisomerase IV (the principal target in Gram-positive bacteria) [2]. Isatin (**2**) is an important endogenous compound present in many organisms. Some isatin derivatives showed excellent anti-tuberculosis activity [5-7].

Simply increasing the lipophilicity may be rendering fluoroquinolones more capable to permeate various biomembrane for improving their permeation properties toward mycobacterial cell membrane. Several series of fluoroquinolones methyl/ethyl isatin derivatives (Fig. 1) with a remarkable improvement in the lipophilicity have been synthesized and evaluated their antituberculosis activity [7-9]. In addition, the ciprofloxacin methylene isatin hybrid **1** (Fig. 2, MIC: 1.39 nM) was more active than moxifloxacin (MXFX, MIC: 1.94 nM) and the parent ciprofloxacin (MIC: 6.04 nM) against *M. tuberculosis* H37Rv, and its cell toxicity (IC_{50}: >111.29 nM) was far less than the references (IC_{50} > 15.58 and > 30.21 nM for moxifloxacin and ciprofloxacin, respectively); hybrid **1** decreased the bacterial load in spleen tissue (mean CFU of 6.08) with 0.76-log10 protections and was considered to be moderately active in reducing bacterial count in spleen.

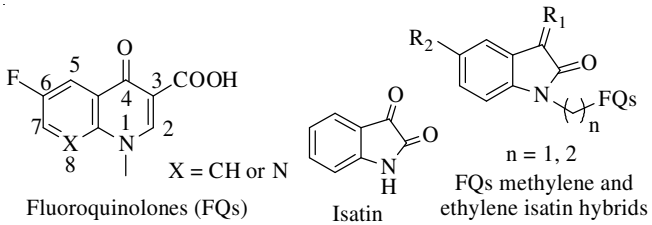

Fig. 1. Chemical structures of fluoroquinolones, isatin and fluoroquinolones methylene and ethylene isatin derivatives

Inspired by the above research results, we designed and synthesized a series of novel ciprofloxacin acetyl isatin

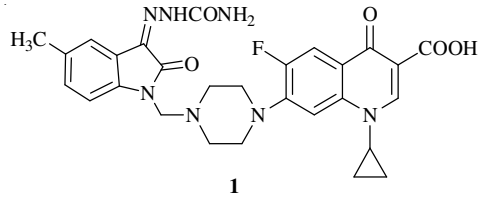

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Fig. 2. Ciprofloxacin methylene isatin **1**

derivatives with remarkable improvement in the lipophilicity compared with the parent ciprofloxacin and evaluated their *in vitro* antimycobacterial activity against *M. smegmatis* and *M. tuberculosis* $H_{37}Rv$.

EXPERIMENTAL

All chemical reagents were of analytical grade and obtained from commercial suppliers and used without further purification unless otherwise noted. Analytical thin layer chromatography (TLC) was performed on glass-backed Analtech Uniplate silica gel plates. ^1H NMR spectra were obtained on a Bruker AVANCE III 400 MHz spectrometer in DMSO-d_6 or $CDCl_3$ using tetramethylsilane (TMS) as an internal standard.

Synthesis: The intermediates and the targets were synthesized by the reported methods [10]. A solution of **1a** or **1b** (10 mmol) in chloroacetyl chloride (100 mmol) was heated to reflux for 5 h. Then the reaction mixture was chilled and diluted with ether (20 mL). The precipitate formed was filtered, washed with cooled ether (2 × 10 mL), dried *in vacuo* and crystallized in ethanol (50 mL) to give intermediate **2a** or **2b**.

A mixture of **2a** or **2b** (10 mmol), ciprofloxacin (10 mmol), anhydrous K_2CO_3 (60 mmol) in DMF (100 mL) was stirred at 50 °C overnight. After removal of the solvent with roto-vap, water (500 mL) was added to the concentrate and the water solution was stirred at room temperature for 10 min, followed by filtration to obtained the solid crude product, which was purified by a silica gel column eluted with DCM:MeOH = 20:1 to give the targets **3a** or **3b**.

To a solution of the corresponding amine hydrochloride (1.0 mmol) and $NaHCO_3$ (0.95 mmol) dissolved in water (10 mL) was added a solution of the targets **3a** or **3b** in EtOH (10 mL) at room temperature. The reaction mixture was stirred at the 40 °C for 12 h and then filtered. The solid obtained was washed with water and EtOH and then purified by a silica gel column with eluted with DCM:MeOH = 20:1 to give the targets **3c-j**.

Determination of MIC: The ciprofloxacin acetyl isatin derivatives (**3a-j**) were tested for their *in vitro* antimycobacterial activity against *M. smegmatis via* serial double dilution technique in duplicate and against *M. tuberculosis* H37Rv *via* rapid direct susceptibility test technique. All the test compounds were dissolved in dimethyl sulfoxide and two-fold diluted at concentrations from 256 to 0.125 μg/mL.

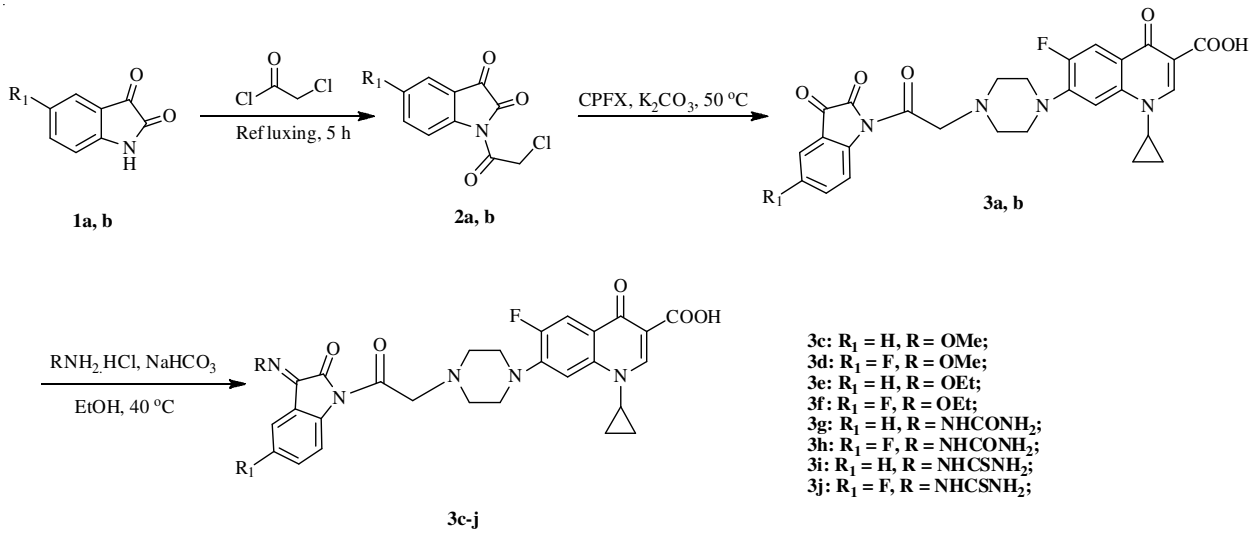
RESULTS AND DISCUSSION

The detailed synthetic route for synthesis of ciprofloxacin acetyl isatin derivatives (**3a-j**) is described in **Scheme-I**. Isatins **1a**, **1b** were amidated with chloroacetyl chloride to give the intermediate **2a** or **2b** according to the reported procedure [10]. Subsequent nucleophilic substitution of intermediate **2a** or **2b** with ciprofloxacin in the presence of K_2CO_3 to give ciprofloxacin acetyl isatin derivative **3a** or **3b**. Finally, condensations of the targets **3a**, **3b** with corresponding substituted amine hydrochlorides provided the other derivatives **3c-j**.

Lipophilicity: The lipophilicity of the synthesized targets **3a-j** and the parent drug ciprofloxacin is indicated in the term of their Clog P values. As shown in Table-1, the lipophilicity of the targets **3a-j** (-0.42 to 2.25, statistically significant at $p < 0.001$ using t test) is much more than ciprofloxacin (-0.73). The improvement of lipophilicity may enhance their antimycobacterial activity.

Antimicrobial activity: The acetyl isatin-tethered ciprofloxacin derivatives **3a-j** along with ciprofloxacin, rifampicin and isoniazid as references were screened for their *in vitro* antimycobacterial activity against *M. smegmatis via* serial double dilution technique in duplicate and against *M. tuberculosis* $H_{37}Rv$ *via* rapid direct susceptibility test technique.

The MICs of ciprofloxacin acetyl isatin derivatives **3a-j** along with ciprofloxacin, rifampicin and isoniazid as references are presented in Table-1. These data indicate that all the compoundeds showed considerable potency in inhibiting the growth of *M. smegmatis* (MIC: 2-32 μg/mL), though less potent



1a, b **2a, b** **3a, b** **3c-j**

Scheme-I: Synthesis of ciprofloxacin acetyl isatin derivatives **3a-j**

TABLE-1
STRUCTURES AND ANTI-MYCOBACTERIAL ACTIVITY OF TARGETS **3a-j**

Target	R_1	R	Clog P	MIC (μg/mL)	
				M. smegmatis	*M. tuberculosis* $H_{37}Rv$
3a	H	OH	-0.42	32	64
3b	F	OH	-0.28	16	128
3c	H	OMe	1.58	16	64
3d	F	OMe	1.72	8	32
3e	H	OEt	2.11	8	16
3f	F	OEt	2.25	8	32
3g	H	$NHCONH_2$	1.02	8	8
3h	F	$NHCONH_2$	1.16	2	32
3i	H	$NHCSNH_2$	1.31	4	16
3j	F	$NHCSNH_2$	1.45	16	32
Ciprofloxacin			-0.73	1	4
Rifampicin				2	8
Isoniazid				4	0.125

than the parent ciprofloxacin (MIC: 1 μg/mL). The activity of **3h** (MIC: 2 μg/mL) was 2 fold more potent than isoniazid (MIC: 4 μg/mL) and is activeat the level to rifampicin (MIC: 2 μg/mL). As for the inhibitory activity against *M. tuberculosis* $H_{37}Rv$, compound **3g** (MIC: 8 μg/mL) was comparable to rifampicin (MIC: 8 μg/mL), but less active than ciprofloxacin (MIC: 4 μg/mL) and isoniazid (MIC: 0.125 μg/mL).

Conclusion

In summary, a series of novel ciprofloxacin acetyl isatin derivatives was designed, synthesized and evaluated for their *in vitro* antimycobacterial activity against *M. smegmatis* and *M. tuberculosis* $H_{37}Rv$. The results indicated that all the targets were active than the parent ciprofloxacin against the tested two strains. All the targets exhibited potent activity against the tested two strains. The most active compound **3h** was found to be 2 fold more potent than isoniazid and comparable to rifampicin against *M. smegmatis*. Compound **3g** was comparable to rifampicin against *M. tuberculosis* $H_{37}Rv$. The result indicated that the lipophilicity of the tested targets is not the sole factor affecting the antimycobacterial activity. Meanwhile, targets containing 5-fluoroisatin did not increase the antimycobacterial activity, when comaped with the corresponding analogs containing an isatin moiety against *M. tuberculosis* $H_{37}Rv$, as seen from the MICs of target **3e** (16 μg/mL) *versus* **3f** (32 μg/mL), **3g** (8 μg/mL) *versus* **3h** (32 μg/mL) or **3i** (16 μg/mL) *versus* **3j** (32 μg/mL).

Antimycobacterial activity of the Schiff's bases (**3c-j**) of derivatives **3a,b** generally improved. The relative contribution of imines of Schiff bases to activity against *M. tuberculosis* $H_{37}Rv$ is as follows: semicarbazone > thiosemicarbazone ≈ ethyl oxime > methyl oxime. Our results enrich the structure-activity relationship of fluoroquinolones isatin derivatives.

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